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Original Article

Invasive urothelial carcinoma exhibiting basal cell immunohistochemical markers: A variant of urothelial carcinoma associated with aggressive features

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ABSTRACT

We characterize invasive urothelial carcinoma (UC) exhibiting urothelial basal cell immunohistochemical markers.

Consecutive invasive UCs were immunostained with CK20 and urothelial basal cell markers, cytokeratin 5 (CK5)/CD44. Immunostaining for CK5 and CD44 was scored as follows: positive for staining of more than 25% thickness of the epithelial nest or epithelium and low for lesser immunoreactivity. Invasive urothelial carcinoma (UC) exhibiting positive CK5/CD44 staining was designated as basal-like UC (BUC).

In this study, of 251 invasive UC (pT1 in 57% and pT2-4 in 43%), BUC accounted for 40% of cases (accounting for most pT2-4 UC) and often presented as non-papillary UC without previous history of UC. In addition, BUC exhibited uniform nuclei with lesser degree of atypia than non BUC and decreased or negative cytokeratin 20 reactivity. Nested and microcystic variants of UC immunohistochemically stained as BUCs. Invasive non-BUCs were often papillary with marked cytologic atypia and pleomorphism, and accounted for most pT1 UC. The rates of perivesical invasion, lymph node and distant metastases were higher for BUC than non-BUC. All nine cases with absent/minimal residual in situ UC in 102 radical cystectomy specimens were from invasive non-BUC.

BUC is distinguished from non-BUC due to this aggressive behavior, distinct immunohistochemical profile, and predominant non-papillary architecture. Our findings are consistent with recent studies identifying a subtype of muscle-invasive UC with molecular expression of basal cell and luminal cell molecular profiles. Our study further supports categorizing invasive UCs into these subtypes with different biological behaviors, possibly contributing to better therapeutic strategies.

Introduction

Urothelium is a special type of mucosal lining; its cytohistological features are characteristic of an epithelium transitional between mono-layered to stratified squamous epithelium. In addition to being an epithelium containing scattered vacuoles in umbrella cells [1], urothelium is also a stratified epithelium. The basally-located cells are recently believed to represent urothelial stem cells and provide an important pool of progenitor cells for more superficial cells and are therefore less differentiated [2–5]. They often display different immunohistochemical features from superficial or luminal cells. Urothelial stem/basal cells are commonly immunoreactive for CD44 and cytokeratin 5 (CK5) [5–7]. In other sites of the body, basal cells are well recognized and are commonly reactive for CK5 and p63 [8–12]. In addition, depending on anatomic site, they are associated with additional immuno-markers such as Ber-EP4 and bcl2 in skin (with diffuse reactivity in basal cell carcinoma and negative reactivity in squamous cell carcinoma), and myoepithelial cell markers in breast or salivary glands [9–12]. In these organs, carcinoma that recapitulates the phenotype of normal basal cells of the native sites is well known, (e.g. basal cell carcinoma of the skin, basaloid squamous cell carcinoma of the upper

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Table 1
Primary antibodies used for immunohistochemistry and associated epitope retrieval methods.

Primary antibody	Manufacturer	Dilution	Antigen retrieval	pH
CK5	Leica Microsystems	Bond ready to use	ERS 2	9.0
CD44v clone 144m-96	Cell Marque	Bond ready to use	Heat induced epitope retrieval	6.0
p63	Leica Microsystems	1/50	ERS 1	6.0
34bE12	Dako	1:100	ERS 1	6.0
BerEP4	Dako	1:400	Dako retrieval solution S1700	6.0
CK20	Leica Microsystems	Bond ready to use	ERS 1	6.0
PIN4	Biocare	Bond ready to use	Heat induced epitope retrieval	6.0
Bcl2	Leica Microsystems	Bond ready to use	Heat induced epitope retrieval	9.0

ERS: Bond Epitope Retrieval System (Leica Microsystems).

aero-digestive tract, tracheo-bronchial system, salivary glands and ano-genital areas). These tumor types share distinct morphology, with hyperchromatic palisading basaloid cells at the periphery of cell nests [13–15]. Some tumors, such as basal-like breast carcinoma, may not display the typical morphologic features as seen in the basaloid squamous cell carcinoma, (such as lack of peripheral hyperchromatic palisading basaloid cells). However, these tumor cells may still be recognized as basal cell-related by their expression of immuno-markers for this cell type such as CK5, muscle-specific actin and S100, and lack immunohistochemical features of luminal cells such as ER, PR and HER2 positivity [16,17,11]. With the exception of cutaneous basal cell carcinoma, basal cell-related tumors are usually associated with aggressive behavior, and with frequent lymph node and haematogenous metastasis [13,17].

Despite the constant presence of basal/stem cells in the urothelium, its neoplastic counterpart has not been well studied. To the best of our knowledge, only two cases of basaloid squamous cell carcinoma of the bladder have been reported [18,19]. Recently, research in our laboratory suggested that a type of urothelial carcinoma (UC) with immunohistochemical features similar to the basal-like carcinoma of the breast exists [20,21]. A recent study also demonstrated the existence of two subtypes of muscle invasive UC similarly to breast carcinoma: basal and luminal subtypes of muscle-invasive bladder cancer [22] Here, we evaluate the expression of markers for urothelial basal/stem cells in a large number of unselected UC and propose that those UCs displaying this profile would be diagnosed as basal-like urothelial carcinoma (BUC).

We also investigated the status of a papillary architecture in BUCs. It is well known that non-papillary high grade UC (UC in situ) is the prevalent precursor of invasive UC [23]. In this study, we focus the investigation on the correlation of muscle invasive UC with: (a) the status of papillary architecture versus flat in situ carcinoma, (b) the expression of CK5 and CD44 in invasive components of UC. Due to the complexity of the association of invasive UC with the intraurothelial neoplastic lesions, we limit our study to the invasive component. Details of intraurothelial neoplastic lesions not associated with invasive UC will be characterized in a separate study [25].

Material and methods

Two hundred and fifty-one consecutive cases of invasive UC from cystectomy specimens or transurethral resection of bladder tumors (TURBT) without significant squamous or glandular differentiation (less than 5% of tumor) were reviewed. UCs were divided into pT1 UC and pT2-4 UC by using the American Joint Commission on Cancer (AJCC/TNM 2010) staging system. Each group was then sub-categorized into papillary and non-papillary types depending on the appearance of the intraepithelial/non-invasive component of the tumor. Surface neoplastic urothelial projections associated with thick stromal and fibrovascular cores were considered as reactive papillary hyperplasia and micro-papillary cell groups on the surface were considered as non-papillary. Reactive atypia, flat urothelial dysplasia and urothelial carcinoma in situ were diagnosed by using criteria of flat intraurothelial lesions [23–25]. Invasive UC is classified as papillary invasive UC if: (a) papillary component accounts for more than 10% of the surface urothelium, or (b) the tumor was associated with papillary UC within a prior period of 6 months. Pathological stage of the carcinoma and lymph node and haematogenous metastatic status were assessed by reviewing slides and the clinical charts. Cystectomy specimens having less than 3 foci of CIS measuring less than 1 mm in diameter were arbitrarily considered as having insignificant residual.

Representative sections from formalin-fixed and paraffin-embedded blocks were submitted for immunostaining using the BOND-MAX automated system (Leica MicroSystems, Richmond Hill, ON). Immunostaining was performed for the following markers (Table 1): CK5, CK20, CD44, p63, high molecular weight cytokeratin 34bE12, bcl-2, EP4, composite of p63 + high molecular weight cytokeratin + alpha-methylacyl-CoA racemase (combined antibody PIN4). Immunostaining for CK5 and CD44 was scored as high for staining of more than 25% thickness of the epithelial nest or epithelium (BUC) and low for lesser immunoreactivity (non-BUC).

These scores were correlated with the histopathological and clinical features including tumor stage and metastatic status. Clinical follow-up ranged from 6 months to 3 years. A retrospective review of the previous tumor resections (up to 6 years) was also performed.

Statistical analysis was performed using SISA software. A *p*-value of less than 0.05 was considered statistically significant.

Results

For a period of 3 years, there were a total of 251 cases of invasive UC. Patients' ages ranged from 46 to 92 years; the male/female ratio was 3:1. UC of pT1 accounted for 143 patients (or 57% of all

Table 2
UC stratified according to level of invasion, status of papillary architecture and CK5 reactivity surgically treated with TURBT only versus radical cystectomy.

Group	Total	Non-papillary (RC)	Papillary (RC)
pT1 (143 cases or 57%)			
BUC	24	5 (2)	19 4)
Non-BUC	119	9 (3)	110 (4)
pT2-4 (108 cases or 43%)			
BUC	77 [54]	73 (69)	4 3)
Non-BUC	31 [16]	26 (13)	5 (3)
Total	251	113 (87)	138 (15)

CK5: mmunostaining for cytokeratin 5, RC: radical cystectomy, UC: urothelial carcinoma.
(): number of cases treated with radical cystectomy. The remaining cases: TURBT only.
[]: number of cases of pT3 or higher.